DEVELOPMENT OF A FUNCTIONALIZED MCM-41 BIOSENSOR MODIFIED WITH SELENIUM NANOPARTICLES FOR GLUCOSE DETERMINATION

NURUL ASYIKIN BINTI KAMARUZAMAN

A thesis submitted in fulfillment of the
requirements for the award of the degree of
Master of Science (Chemistry)

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Special dedications to

My beloved:

Dad and Mum..... Kamaruzaman Abd Rasid and Nor Arbaayah Sharif

Brothers..... Nor Zaidy, Nor Zaid, Nor Aizat and Nor Hazmi

Sisters in Law.... Sabrina Bt. Sabri and Noor Shahidah Satar

My respected project supervisor

Prof. Alias Mohd Yusof

Co-Supervisors

Prof. Noor Aini Abdul Rashid

Assoc. Prof. Dr. Abdull Rahim Mohd Yusoff

And my dearest friends.....

Thank you for always helping and supporting me

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PREFACE

In this work, several reports have been published and presented in the conferences and journals, as listed below:

1. Alias Mohd Yusof, Abdull Rahim Mohd Yusoff, Noor Aini Abdul Rashid, Shafinaz Shahir and Nurul Asyikin Kamaruzaman. *Effect of Temperature and Calcination Time in Characterizations of MCM-41*. Second International Conference and Workshops on Basic and Applied Sciences and Regional Annual Fundamental Science Seminar 2009 (ICORAFSS 2009), The ZON Regency Hotel, Johor Bahru, Johor, 2-4 June 2009.

2. Alias Mohd Yusof, Abdull Rahim Mohd Yusoff, Noor Aini Abdul Rashid, Shafinaz Shahir and Nurul Asyikin Kamaruzaman. *Synthesis of selenium particles in the presence of MCM-41 at different aging temperature.* 10th Asian Conference on Analytical Sciences 2009 (ASIANALYSIS X). PWTC, Kuala Lumpur, 11th – 13th August 2009.

3. Alias Mohd Yusof, Abdull Rahim Mohd Yusoff, Noor Aini Abdul Rashid, Shafinaz Shahir and Nurul Asyikin Kamaruzaman. *Synthesis and Characterization of Selenium Nanoparticles Induced by Ultrasonication Irridiation with Ascorbic Acid as Reducing Agents.* Faculty Science Post-Graduate Conference (FSPGS) 2010, UTM Skudai, Johor, 2010.

4. Alias Mohd Yusof, Abdull Rahim Mohd Yusoff, Noor Aini Abdul Rashid, Shafinaz Shahir and Nurul Asyikin Kamaruzaman. *Glucose Oxidase-Functionalized-Selenium Nanoparticles-Mesoporous Silica as a Glucose Biosensor (HyperSiliSel)*. 12th Industrial Art and Technology Exhibition 2010

(INATEX 2010). 5-7 August 2010, Dewan Sultan Iskandar, Universiti Teknologi Malaysia.

5. Alias Mohd Yusof, Abdull Rahim Mohd Yusoff, , Shafinaz Shahir, Shakil Mohammad Arif, Nurul Asyikin Kamaruzaman and Nik Ahmad Nizam Nik Malek. *A Cholesterol Biosensor Based on Cholesterol Oxidase Immobilized onto Functionalized Mesoporous Silica (CHOL-E-SENSE)*. 12th Industrial Art and Technology Exhibition 2010 (INATEX 2010). 5-7 August 2010, Dewan Sultan Iskandar, Universiti Teknologi Malaysia.

6. Silver Award in 12th Industrial Art and Technology Exhibition 2010 (INATEX 2010). 5-7 August 2010, Dewan Sultan Iskandar, Universiti Teknologi Malaysia for research project of *A Cholesterol Biosensor Based on Cholesterol Oxidase Immobilized onto Functionalized Mesoporous Silica (CHOL-E-SENSE)*.

ABSTRACT

A novel biosensor for glucose determination had been developed in this study. Glucose biosensor is a good example of a commercial biosensor. It uses glucose oxidase (GO_x), a redox enzyme to break down glucose to hydrogen peroxide and coupled with amperometric detection. For the construction of such a biosensor, a broad applicable method in the immobilization of enzyme is critically needed. One way to go about this is through the development of a new carrier such as nanoparticles. Here, a silica-based material, MCM-41, was used as enzyme support material, functionalized and modified with selenium (Se) nanoparticles and then fabricated into a biosensor. MCM-41 was synthesized and characterized to analyze the structural, morphological, elemental and physicochemical characteristics. It was later confirmed that MCM-41 of high purity and high surface area was synthesized. Pristine and unmodified MCM-41 may not be suitable as enzyme support material because it cannot provide the necessary sites for enzyme attachment. Therefore, two types of MCM-41 supports were produced: *f*-MCM-41 and *f*-SeMCM-41. The first one, *f*-MCM-41 was modified for immobilization of GO_x and minimum leaching of the enzyme by functionalizing with amino groups using 3-aminopropyl triethoxysilane (APTES), followed by attachment of aldehyde group using glutaraldehyde. The latter, *f*-SeMCM-41, was co-functionalized with amino group during selenium nanoparticles (SNs) attachment onto the silicate framework to increase sensitivity and electrical conductivity for a better response. The product was then functionalized with glutaradehyde. Selenium nanoparticles (SNs) were successfully synthesized using a simple, cost effective and non-hazardous procedure where selenious acid was reduced using ascorbic acid, ultrasonicated and aged for 24h. Characterization showed that SNs of hexagonal crystalline type with high purity of more than 95.0% was produced. The incorporation process of SNs onto MCM-41 did not alter the structure of MCM-41 or even the SNs as observed by X-Ray Diffraction Spectroscopy (XRD). It was found that GO_x-*f*-Se-MCM-41 was more efficient than GO_x-*f*-MCM-41 as determined by the specific activity of GO_x immobilized onto them. The optimum pH for immobilization of GO_x onto both functionalized MCM-41 and Se-MCM-41 was determined to be pH 6.0 and the optimum initial GO_x concentration was 2.0 mg/mL. GO_x-*f*-MCM-41 and GO_x-*f*-Se-MCM-41 were used in the fabrication of carbon paste electrodes (CPE) and the efficiency examined. GO_x-*f*-Se-MCM-41/CPE electrode was more sensitive and efficient as compared to GO_x-*f*-MCM-41/CPE electrode, as evaluated using cyclic voltammetry. GO_x-*f*-Se-MCM-41/CPE can detect very low range of glucose between 3.69 μM to 16.25 μM. Normal human glucose level is between 3.3 to 3.8 mM but this biosensor can detect much lower levels making it an excellent biosensor for clinical and industrial use. Hence, the newly developed functionalized MCM-41 support with immobilized glucose oxidase with Se attached to it, GO_x-*f*-Se-MCM-41/CPE offers the potential exploitation of a suitable glucose biosensor.

ABSTRAK

Suatu biosensor baru telah dibangunkan dalam kajian ini bagi penentuan glukosa. Biosensor glukosa adalah contoh yang terbaik untuk biosensor komersial. Ia menggunakan oxidasa glukosa (GO_x), enzim redoks bagi glukosa untuk memecahkannya kepada hidrogen peroksida dan digabungkan dengan pengesanan amperometrik. Satu kaedah yang mudah bagi menyahgerakkan enzim amat diperlukan untuk pembinaan biosensor. Salah satu cara adalah melalui pembangunan pengangkut baru seperti nanopartikel. Di sini, bahan berasaskan silika, MCM-41 telah digunakan sebagai bahan sokongan enzim, difungsikan dan diubahsuai dengan selenium (Se) nanopartikel (SNs) yang kemudiannya direka menjadi biosensor. MCM-41 telah disintesis dan dicirikan untuk menganalisis struktur, morfologi, unsur dan ciri-ciri fizikokimia. MCM-41 yang telah disintesis disahkan mempunyai ketulenan yang tinggi dan luas permukaan yang besar. MCM-41 yang asli dan tidak diubahsuai tidak sesuai sebagai bahan sokongan enzim kerana ianya tidak dapat menyediakan tapak yang diperlukan untuk sokongan enzim tersebut. Oleh itu, dua jenis MCM-41 telah dihasilkan: *f*-MCM-41 dan *f*-Se-MCM-41. Yang pertama, *f*-MCM-41 telah diubahsuai bagi pergerakan GO_x menunjukkan larut lesap enzim yang minimum apabila difungsikan dengan kumpulan amino menggunakan 3-aminopropil trietoksisilana (APTES), diikuti oleh pelekatan kumpulan aldehid menggunakan glutaraldehid. Kedua, *f*-SeMCM-41, difungsikan seperti di atas tetapi dengan pengubahsuaian selanjutnya melalui penggabungan SNs ke dalam rangka kerja silikat untuk meningkatkan kepekaan dan kekonduksian elektrik bagi tindak balas yang lebih baik. Kemudian, produk telah difungsikan dengan glutaraldehid. SNs telah berjaya disintesis dengan menggunakan kaedah yang mudah, kos yang efektif dan tidak berbahaya di mana asid selenious telah diturunkan dengan menggunakan asid askorbik, diultrasonik dan disimpan di tempat gelap selama 24 jam. Pencirian menunjukkan bahawa SNs jenis kristal heksagon dengan ketulenan yang tinggi melebihi 95.0% telah dihasilkan. Proses penggabungan SNs ke MCM-41 tidak mengubah struktur MCM-41 atau SNs seperti yang ditunjukkan oleh Pembelauan sinar-X (XRD). Ia mendapati bahawa GO_x-*f*-Se-MCM-41 adalah lebih berkesan daripada GO_x-*f*-MCM-41 seperti yang dihitung oleh aktiviti spesifik GO_x yang dinyahgerak ke atas bahan sokongan ini. pH optimum untuk pergerakan GO_x ke atas kedua-dua MCM-41 atau Se-MCM-41 difungsi telah ditetapkan pada pH 6.0 dan kepekatan awal GO_x optimum adalah 2.0 mg/mL. GO_x-*f*-MCM-41 dan GO_x-*f*-Se-MCM-41 telah digunakan dalam fabrikasi elektrod pasta karbon (CPE) dan kecekapannya dikaji. Elektrod GO_x-*f*-Se-MCM-41/CPE adalah lebih peka dan berkesan berbanding GO_x-*f*-MCM-41/CPE elektrod, seperti yang dianalisis dengan menggunakan voltammetri kitaran. GO_x-*f*-Se-MCM-41/CPE boleh mengesan glukosa yang sangat rendah antara 3.69 μM ke 16.25 μM. Paras glukosa manusia normal adalah di antara 3.3-3.8 mM tetapi biosensor ini dapat mengesan pada tahap yang lebih rendah menjadikannya biosensor yang sangat baik untuk kegunaan klinikal dan industri. Oleh itu, MCM-41 sokongan yang baru dibangunkan telah difungsikan dengan GO_x dinyahgerakkan dengan Se terlekat padanya, GO_x-*f*-Se-MCM-41/CPE, berpotensi untuk dieksploitasi sebagai biosensor glukosa.

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LIST OF ABBREVIATIONS

AA	Ascorbic acid
ADA	American Diabetes Association
AOT	Sodium Bis(2-ethylhexyl) Sulfosuccinate
AP	Acetaminophen
APTES	3-aminopropyltriethoxysilane
APTMS	3-aminopropyltrimethoxysilane
a-Se	Amorphous Selenium
Au/GNPs-SBA-15/IO_4^- oxidized-GOD	Gold/Gold Nanoparticles-SBA-15/ Metaperiodate Ion Oxidized- Glucose Oxidase
Au/H_2N-SBA-15/IO_4^--oxidized-GOD	Gold/Amine-SBA-15/ Metaperiodate Ion Oxidized-Glucose Oxidase
Au/SWNT/GOD/PPy	Gold-Single Wall Nanotubes/ Glucose Oxidase/Polypyrrole
Au/SWNT/GOD-HRP/PPy	Gold/Single-Walled Carbon Nanotubes/Glucose Oxidase/Horseradish Peroxidase/Polypyrrole
Au/SWNT/HRP-PPy/GOD-PPy	Gold/Single Wall Nanotubes/ Horseradish Peroxidase/Polypyrrole/Glucose Oxidase/Polypyrrole
BSA	Bovine Serum albumin
CM100B	*Bacillus cereus*
CTAB	Cetyltrimethylammonium Bromide
CV	Cyclic voltammetry
d_{100}	Plane 100
d_{110}	Plane 110
D_{200}	Plane 200
D_{210}	Plane 210

DNA	Deoxyribonucleic Acid
DPCSV	Differential Pulse Cathodic Stripping Voltammetry
DTA	Differential Thermal Analysis
DTT	Dithiothreitol
E_{acc}	Accumulated potential current
EDX	Energy Dispersive X-Ray Spectroscopy
E_i	Initial potential current
$Enzyme_{ox}$	Enzyme oxidized
$Enzyme_{red}$	Enzyme reduced
f- Se-MCM-41	Functionalized-Selenium Nanoparticles-Mobil Crystalline Materials No. 41
Fe-MCM-41	MCM-41 Modified with Iron.
FESEM	Field Emission Scanning Electron Microscopy
f-MCM-41	Functionalized- Mobil Crystalline Materials No. 41
FSM-16	Folded-Sheet Mesoporous Material
FTIR	Fourier Transform Infrared Spectroscopy
G-CdS	Graphene-Cadmium Sulphur
GNPs	Gold nanoparticles
GO_x	Glucose Oxidase
GO_x-*f*- MCM-41/CPE	Glucose Oxidase-Functionalized Mobil Crystalline Materials No. 41/Carbon Paste Electrode
GO_x-*f*-MCM-41	Glucose Oxidase-Functionalized- Mobil Crystalline Materials No. 41
GO_x-*f*-Se-MCM-41	Glucose Oxidase-Functionalized Selenium Nanoparticles- Mobil Crystalline Materials No. 41
GO_x-*f*-Se-MCM-41/CPE	Glucose Oxidase-Functionalized Se-Mobil Crystalline Materials No. 41/Carbon Paste Electrode
HDP	Hydrodeporphirinization
HDTMA	Hexadecyltrimethylammonium Bromide
HMDS	Hexamethyldisilazane
ICP-MS	Inductively Coupled Plasma Mass Spectroscopy
IDDM	Insulin Dependent Diabetes Mellitus / Juvenile Diabetes
LOD	Correlative of Determination

MCF	Mesostructured Cellular Foam
MCM-41	Mobil Crystalline Materials No. 41
MCM-41-A	Amino-Mobil Crystalline Materials No. 41
MCM-41-as	As-synthesized Mobil Crystalline Materials No. 41
MCM-41-C	Calcined Mobil Mobil Crystalline Materials No. 41
MCM-41-C-1d	One day calcined Mobil Crystalline Materials No. 41
MCM-41-C-2d	Two day calcined Mobil Crystalline Materials No. 41
MCM-41-C-3d	Three day calcined Mobil Crystalline Materials No. 41
MCM-48	Mobil Crystalline Materials No. 48
MCM-50	Mobil Crystalline Materials No. 50
MOX	Malaysian Oxygen Berhad
MPTMS	3-mercaptopropyltrimethoxysilane
MWCNTs	Multi-walled carbon nanotubes
NIDDM	Non Insulin Dependent Diabetes Mellitus
PDF	Powder Diffraction File
POD	peroxidase type II from Horseradish
Pt/MCM-41	Platinum Nanoparticles/Mobil Crystalline Materials No. 41
Pt/sulfonated-MWCNTs	Platinum/sulfonated multi-walled carbon nanotubes
PTFE	Polytetraflouroethylene
PtMCWNTs	Platinum multi-walled carbon nanotubes
R^2	Correlative of determination
SBA-15	Santa Barbara Amorphous
Se-MCM-41	Selenium Nanoparticles-Mobil Crystalline Materials No. 41
SNs	Selenium Nanoparticles
ß-monoclinic Se	Black crystalline Selenium (Se_8 rings)
t_{acc}	Accumulated time
TEM	Transmission Electron Microscopy
TGA	Thermal Gravimetric Analysis
TrxRs	Thioredoxinreductase
t-Se	Trigonal Selenium

UA	Uric acid
UV-Vis	Ultraviolet-visible Spectroscopy
XRD	X-ray Diffraction Spectroscopy
α-monoclinic Se	Red crystalline Selenium (Se_8 rings)
ΔA	Absorbance changes
θ	Theta
Φ	Phi

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Diabetes is one of the critical diseases that are characterized by elevated glucose levels because the body cannot produce sufficient insulin or the insulin becomes resistant in regulating glucose. Worldwide, diabetes is a serious public health problem that will impact health care financing (Vinicor, 1998; Narayan *et al.*, 2000; Zhang *et al.*, 2010). Several Western countries reported that diabetic patients have increased their medical expenditure compared to individuals without diabetes from the severe macrovascular and microvascular complications associated with diabetes (Rubin *et al.*, 1994; Kangas *et al.*, 1996; Selby *et al.*, 1997; Brown *et al.*, 1999; Oliva *et al.*, 2004).

Latest data reported by the American Diabetes Association (ADA) mention that in the United States, 17 million people or 6.2% of the population were diagnosed as diabetic with 35% (5.9 millions) of these cases undiagnosed. Each year about 12,000 to 24,000 new cases of adult blindness caused by diabetes were recorded. In 1999, more than 114,000 cases of diabetes-related dialysis or transplantation that refers to end-stage renal disease were recorded. While between 1997 and 1999, 82,000 of diabetes-related amputations were recorded as non-traumatic lower extremity amputation (Hirsch, 2002).

In 1995, the number of adults with diabetes was around 135 million and this figure will rise to 300 million in the year 2025. This number, along with the

discovery of a million new cases yearly makes diabetes one of the most important national health issues that we must consider as serious and it is getting worse in developing countries because diabetes rates are increasing faster and is expected to increase 170% from 1995 to 2025 (King *et al.*, 1998). This problem lies in our lifestyle today as a result of poor nutrition intake and a sedentary lifestyle.

The inability of the body to control blood glucose levels can lead to acute and chronic complications. Hypoglycemia is a condition where blood glucose (glycemia) level rapidly drops to the lowest level causing mental confusion, convulsions and leading to coma or death. Chronically increased levels of glucose in the blood (chronic hyperglycemia) and an abnormally high level of proteins covalently bind with glucose (glycation or glycosylation) contributing to long-term microvascular and macrovascular complications (Lieberman and Marks, 2009).

Biosensor systems were used to detect many biological compounds especially in the field of biotechnology based on enzyme-substrate interactions. One of the applications of biosensor was to determine the levels of glucose in human body using glucose biosensor. Many researchers have developed new biosensors in order to increase sensitivity. Recently, the most popular biosensors reported are those employing redox enzyme coupled with amperometric detection because this method has the advantages of being more stable, inexpensive, simple to operate, disposable and suitable for real time detection (Chen *et al.*, 2002; Mitala Jr and Michael, 2006; Dai *et al*., 2007; Sun *et al.*, 2007). Amperometric biosensors possess linear concentration dependence and measure changes in the current on the working electrode due to the direct oxidation of the products of a biochemical reaction in direct or indirect system. One of the key factors in the building of a reliable biosensor is the development of better techniques for immobilization of enzymes. Thus, new immobilization methods and supports are highly desired to improve the analytical capacities of sensor devices to enhance the properties such as reusability, operational stability, recovery and self life (Arai *et al.*, 2006; Jena and Raj, 2006; Park *et al.*, 2006; Shen *et al.*, 2007; Yogeswaran and Chen, 2008; Chen *et al.*, 2009; Vidotti *et al.*, 2011).

Electrochemical sensors have improved the performance of the conventional analytical tools, eliminated slow preparation, reduced the uses of expensive reagents and provided low cost analytical tools. Electrochemical sensors have certain advantages over the conventional analytical instruments such as inexpensive, portable and simple to operate. On the other hand, they also have some limitations where there are electrochemically active interferences in the sample, weak long-term stability and troublesome electron transfer pathways. However, electrochemical sensors were always applied in clinical diagnosis, environmental monitoring and food analysis.

Selenium nanoparticles (SNs) exhibit not only photoelectric, semiconductor and X-ray sensing properties, but also biological activity and good adsorptive ability due to their interaction between the SNs and N-H, C=O, COO- and CN- groups of the protein (Smith, and Cheatham, 1980; Ohara *et al.*, 1997; Gao *et al.*, 2002; Zhang *et al.*, 2008; Barnaby *et al.*, 2010). Hence, the SNs can be easily attached to support or enzyme and can function as a sensor that provide good amperometric signal. Many researchers have studied and synthesized stable SNs in polymer matrices (Kopeikin *et al.*, 2003a, b; Zhang *et al.*, 2007) and polysacchrides (Gao *et al.*, 2002; Zhang *et al.*, 2004a) such as chitosan (Zhang *et al.*, 2004b). MCM-41 have been found to be an exciting candidate as support for SNs compared to polymer and polysaccharides because of their uniform and adjustable pore size, defined pore and cage system, high surface area, shape and charge selectivity, high thermal stability, resistance to biodegradation and opened pore structures.

Glucose oxidase (GO_x) is a flavoprotein which catalyzes the oxidation of β-D-glucose by molecular oxygen to δ-gluconolactone, which subsequently spontaneously hydrolyzes to gluconic acid and hydrogen peroxide (Arica and Hasirci, 1993; Zoldak *et al.*, 2004).

$$\beta\text{-D-glucose} + H_2O + O_2 \xrightarrow{GO_x} \text{D-gluconic acid} + H_2O_2 \qquad \text{Equation 1.1}$$

$$H_2O_2 \xrightarrow{\text{catalase}} \tfrac{1}{2}\, O_2 + 2\, H_2O \qquad \text{Equation 1.2}$$

Industrially, it is used in the removal of glucose or oxygen from food products and also applied in the production of gluconic acid (Szajani *et al.*, 1987; Ekinci *et al.*, 2007). The most important application of glucose oxidase was as a molecular diagnostic tool. The enzyme was used in biosensors for the quantitative determination of D-glucose in samples such as body fluids, foodstuffs, beverages, and fermentation products (Zoldak *et al.*, 2004). When it is applied in the voltammetry technique as a biosensor, the reaction of glucose oxidase at electrode is shown in Equation 1.3 and 1.4.

$$\text{Glucose} + O_2 \xrightarrow{GO_x} H_2O_2 + \text{Gluconic Acid} \qquad \text{Equation 1.3}$$

$$H_2O_2 \xrightarrow{\text{Electrode}} 2e^- + O_2 + 2\,H^+ \qquad \text{Equation 1.4}$$

1.2 Problem Statements

Concern about health and nutrition problems is worldwide, and the detection of glucose by glucose biosensor has attracted a high degree of interest especially amongst diabetics. There are many types of glucose biosensors in the market but it is mostly too expensive. Thus, the development of a new glucose biosensor with better accuracy, high sensitivity and lower cost are needed. Today, mesoporous materials such as MCM-41, MCM-48, MCF and SBA-15 have received much attention because of their wide use in many absorbent and catalytic reactions. In addition, mesoporous materials are easy to synthesize. But mesoporous materials have their limitation in that they lack active sites for bonding with enzyme. They have to be modified to make their surface more conducive for enzyme attachment. In order to make these materials as potentially better catalysts, incorporation of metal centers such as Al, V, Fe and Mn in the silicate framework are necessary (Ozyilmaz *et al.*, 2005).

Selenium nanoparticles (SNs) was chosen not only by their unique photoelectric, semiconducting and X-ray-sensing properties but also for its biological activity and good adsorptive ability due to the interactions between the SNs and NH,

C=O, COO- and C-N groups of the proteins (Smith and Cheatham, 1980; Ohara *et al.*, 1997; Gao *et al.*, 2002; Zhang *et al.*, 2008; Barnaby *et al.*, 2010). So, it can be used as a new rectifier for the component of redox enzymes based on biosensors (Zhang *et al*., 2004b) hence chosen in the development of a more sensitive glucose biosensor

1.3 Objectives of Study

The objectives of this work are as follows:

i. To synthesize and characterize MCM-41 and selenium nanoparticles (SNs).
ii. To modify MCM-41 with the incorporation of SNs into MCM-41 in the presence of amino group and its characterization.
iii. To functionalize MCM-41 and Se-MCM-41 with amino group followed by aldehyde groups and their characterization.
iv. To optimize the immobilization of glucose oxidase enzyme (GO_x) onto functionalized MCM-41 and Se-MCM-41.
v. To study the electrochemical properties of the GO_x-*f*-MCM-41 and GO_x-*f*-Se-MCM-41 as glucose biosensor.

1.4 Scope of Study

The scope of study covers the synthesis of MCM-41 and SNs, followed by the modification of MCM-41 with SNs in the presence of amino group (Se-MCM-41) followed by functionalization with aldehyde group (*f*-Se-MCM-41). In order to make comparison, MCM-41 was also functionalized with amino group followed by the aldehyde group. The characterization of its structural and chemical characteristics of MCM-41, SNs, Se-MCM-41 and functionalized MCM-41 (*f*-MCM-41) and Se-MCM-41 (*f*-Se-MCM-41) were investigated. The optimum conditions for the

immobilization of GO_x enzyme onto *f*-MCM-41 and *f*-Se-MCM-41 were investigated. The specific activity and percentage of GO_x bound onto *f*-MCM-41 (GO_x-*f*-MCM-41) and *f*-Se-MCM-41 (GO_x-*f*-Se-MCM-41) were determined. Then, the GO_x-*f*-MCM-41 and GO_x-*f*-Se-MCM-41 were applied as the working electrode in the electrochemical analysis (voltammetry) to determine which of these two types of electrode was suitable and feasible for the development of glucose biosensor. Particular attentions were given on the scope of electrochemical analysis which were focused to differential pulse sweep and cyclic voltammetry analysis.

1.5 Significance of Study

Glucose analysis is very important in food industry for quality control purposes, fermentation and most importantly in clinics and hospitals for diagnosing diabetic patients using glucose biosensor. This method usually involves an enzyme which is very specific for glucose and is not easily interfered by other sugars present. Due to its high selectivity towards ß–D-glucose, the enzymatic method for glucose determination employs the use of glucose oxidase (GO_x). It is one of the most robust enzymes which can withstand extreme pH, ionic strength and temperature compared with other enzymes. Thus, it allows less stringent conditions during the manufacturing process and also provides relatively care-free storage which makes it more suitable for the glucose biosensors especially for home-users.

This work also deals with the modification of MCM-41 with SNs to investigate the development of a better working electrode in glucose biosensor compared to pristine MCM-41. The disadvantage of using pure mesoporous material for immobilization of enzymes is the absence of active sites in their matrices which limit the quantity of enzymes bound onto it. Besides that, incorporation of metal centers such as SNs in the silicate framework is necessary in order to increase the performance of glucose biosensor due to its semiconductor and antioxidant properties. With the incorporation of metal, Se-MCM-41 can be a suitable material for GO_x loading as well as promote electron transfer between GO_x and the electrodes.

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